Supplemental Material

Supplemental Figure Legends

Figure S1. Validation of mTAIL-seq.

(A) Schematic of experimental procedures. (left) TAIL-seq, (right) mTAIL-seq. Common steps are shown in black color while red indicates method-specific procedures. Blue bars and black bars represent mRNAs and 3′ adpators, respectively. N (random sequence) and T (thymine) shown in 3′ adaptors are abbreviated proportional to the original length. B refers to a biotin.

(B) Ligation efficiency test. 5′ labeled substrates which have various tails (A10, A10U10, and A50) are ligated with denoted 3′ adaptor (mTAIL-seq-1 and mTAIL-seq-2). Blue bar refers to 20 nt heterogeneous sequences (5′-UUUAUUACAGCUCUACCUAG-3′). Black bar represents the 3′ adpator. N (random sequence) and T (thymine) shown in adaptors are abbreviated to the original length. Red arrows indicate the ligated products. Dashed line marks discontinuous lanes from the same gel.

(C) TAIL-seq reads are enriched in the 3′ part of genes. X-axis shows a relative distance between the 5′ end of read 1 and the annotated 3′ end.

(D) Scatter plots showing the correlation between poly(A) tail lengths measured with four different amounts of input RNA from HeLa. R_p refers to Pearson correlation coefficient.

(E) Comparison of poly(A) tail lengths estimated by TAIL-seq and mTAIL-seq. R_p refers

to Pearson correlation coefficient.

(F) Detection of U-tails by mTAIL-seq. (Top) 3′ adaptors used in TAIL-seq and mTAIL-seq are shown in black bars. The nucleotide composition of overhang is denoted in the name (T8, T7A1, and T6A2). Blue bar refers to 3′ end of transcript. (Bottom) Poly(A) tail lengths from 8 nt to 231 nt are pooled in equal-width bins in the logarithmic scale (base 2) (x-axis). The left sides of bins (inclusive) are 8, 11, 15, 20, 26, 34, 46, 61, 81, 108, 144, 192 nt. Uridylation frequency (y-axis) indicates the percentage of mono-U and di-U tails within each length range.

Figure S2. Poly(A) tail length profiles and 3′ uridylation rate of *Drosophila* samples.

(A) 3′ uridylation frequency of mRNAs with short poly(A) tail (5–25 nt) detected by TAIL-seq. Three independent biological replicates from embryos (0–2 hr) and S2 cells are shown along with HeLa and NIH 3T3 (Chang et al., 2014).

(B) Virtual gel image of poly(A) tail length distribution from *Drosophila* early embryos and S2 cell. The total intensity of each bin (intensity multiplied by area) is proportional to read counts and normalized by each lane.

(C) Reproducibility between two biological replicates of mTAIL-seq. R_p refers to Pearson correlation coefficient.

(D) Global distributions of poly(A) tails at three stages in biological replicates. The median poly(A) tail lengths is 60 nt in immature oocytes, 69 nt in mature oocytes, and 66 nt in activated eggs.

(E) Scatter plots showing the correlation between mRNA abundance change and mean poly(A) length change during late oogenesis and egg activation, respectively. For each stage transition, densities of mRNA abundance change and mean poly(A) length change are plotted in upper and right sides of the scatter plot, respectively. R_p refers to Pearson correlation coefficient.

(F) Results of high-resolution poly(A) tail assay (Hire-PAT). The signal intensity is normalized to maximum value at each stage, except for *osk*, the signal of which is fitted into the immature oocyte stage.

Figure S3. Intragenic poly(A) tail length distributions in two replicates.

(A) Heat maps showing the distributions of intragenic poly(A) tail lengths in each group. Poly(A) length is discretized with 20 nt wide bins, and the color intensity indicates the fraction of poly(A) tags for the gene. For each heat map, genes are sorted and reordered by geometric mean of poly(A) length (colored line).

(B) Poly(A) tail distribution of two representative genes from each group is presented as in Fig. 2E.

Figure S4. Changes of poly(A) tail length and mRNA abundance in *wisp* mutants.

(A) Reproducibility between two biological replicates of *wisp* mutant.

(B) Violin plots showing the changes of mRNA abundance in *wisp* mutant at three different

stages (*P <2.2 × 10^{-16}, two-sided Kolmogorov-Smirnov test). Black line refers to the median.

(C) Comparison of poly(A) tail lengths between wild type and *wisp* mutant in each stage as in Fig. 4A. Dashed line marks 1.5-fold reduction. Turquoise dots indicate mitochondria-related genes.

Figure S5. Correlation between poly(A) tail length and translation.

(A) Poly(A) tail lengths of mature oocytes and activated eggs (0–1 hr) are compared to translational efficiency as in Fig. 5A.

(B) A scatter plot showing the correlation between mean poly(A) length changes (from mature oocytes to activated eggs [0–1 hr]) and TE changes as in Fig. 5B.

(C) Violin plots showing the changes in ratio of polysome fractions (≥5 ribosomes) to monosome fractions (40S, 60S, and 80S) between activated eggs and mature oocytes. Polysome profiling and RNA-seq were adopted from Kronja et al. (Kronja et al., 2014). Each color indicates a corresponding group defined in Fig. 3A, and poly(A) length changes of each group are simplified in the bottom panel. Black line represents the median.

Supplemental Table Legends

Table S1. The poly(A) tail lengths and poly(A) tag counts of genes detected by

mTAIL-seq in immature oocytes, mature oocytes, and activated eggs. The list of genes with ≥50 poly(A) tags in both replicates are shown. The poly(A)+ tags indicate reads with longer than 8 nt A tails.

Table S2. The abundance of mRNA species detected by RNA-seq. The list of genes with normalized reads from wild type or *wisp* mutant in each stage are shown.

Table S3. The list of genes belongs to 8 groups.

Table S4. The list of all gene ontology (GO) terms detected in defined groups of poly(A) tail length profiles.

Table S5. The poly(A) tail lengths and poly(A) tag counts of genes detected by mTAIL-seq in *wisp* mutants. The list of genes is shown as in Table S1.

Supplemental Methods

High-resolution poly(A) tail assay (Hire-PAT)

To validate the poly(A) tail length measured by mTAIL-seq, Hire-PAT was carried out as previously described (Chang et al., 2014). Primers used in Hire-PAT are *Tl* forward 5′-CTATGTGTATATCGTCTAAGC-3′; *osk* forward 5′-GTAGCACAGTGTAGAATTCTG-3′; *sop* forward 5′-GGATTGCTACACCTCGGCCCGT-3′.

Polysome profiling analysis

As in the ribosome profiling analysis, deposited libraries were used for analysis

(GSE52799) (Kronja et al., 2014). Sequencing reads were trimmed into 27 nt-long sequences, then filtered with Phred quality score. The *Drosophila* dm6 genome and the *Saccharomyces cerevisiae* genome (Ensembl assembly R64-1-1 release 82) were combined, and gene annotation file was made by merging reduced fly RefSeq and yeast GTF file as previously described (Kronja et al., 2014). Sequencing reads were mapped to the merged genome using RSEM (Li and Dewey, 2011). The top 20 highly expressed yeast genes which were sorted by the highest abundance in the RNP fraction were regarded as an exogenous control group, and fly transcript levels were normalized with RUVg (k=1, (Risso et al., 2014)). To reduce noises, transcripts with <100 normalized reads in fraction which corresponds to ≥10 ribosomes were excluded. For each fly transcript, a percentage in each fraction was calculated by dividing normalized reads by total reads. Percentage recruitment to polysomes (≥5 ribosomes) and monosomes (40S, 60S, and 80S) were calculated and used for comparing a degree of translational activation.

Supplemental References

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Kronja, I., Yuan, B., Eichhorn, S.W., Dzeyk, K., Krijgsveld, J., Bartel, D.P., and Orr-Weaver, T.L. (2014). Widespread changes in the posttranscriptional landscape at the Drosophila oocyte-to-embryo transition. Cell reports *7*, 1495-1508.

Li, B., and Dewey, C.N. (2011). RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC bioinformatics *12*, 323.

Risso, D., Ngai, J., Speed, T.P., and Dudoit, S. (2014). Normalization of RNA-seq data using factor analysis of control genes or samples. Nature biotechnology *32*, 896-902.